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Framework for a Benthic Aquaculture Monitoring Program in the Pacific Region

Cadre pour un programme de surveillance de l'aquaculture en milieu benthique dans la Région du Pacifique

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Abstract

Benthic monitoring programs designed to detect environmental change require the following aspects to be defined: 1) spatial boundaries and temporal fluctuations of impact zone; 2) reference zones for each impacted substrate or habitat type; 3) cause and magnitude of environmental change; and 4) future predictions of impact trends.

In order to define the spatial extent of an impact zone, comparisons of before and after conditions and/or control and impact conditions should be considered. It is important that the baseline data in support of "before" conditions be collected in regions that will receive impact, while date characterizing "control" conditions be collected in regions that will remain uninfluenced by far-field knowledge of the hydrography, substrate, bathymetry, and habitat characteristics of the impact site in order to provide a proper comparison of the control and impact environments. The selection of the appropriated physical, chemical and biological parameters to be incorporated into a monitoring program will largely influence the capability to detect environmental change. Properties to be measured should include: 1) tracers of waste materials (i.e. Zinc) altered chemical and physical parameters resulting from waste input (i.e. Sulphides/Redox) and 3) biological responses to the direct waste inputs and indirect by-products. Further research providing practical examples of data that compares spatial and temporal variance at reference/impacted sites within the Pacific Region sis required in order to create a hierarchical ranking of monitoring parameters according to their ability to detect ecological impact.

Résumé

Pour mettre en œuvre des programmes de surveillance des modifications environnementales du milieu benthique, il faut définir les aspects suivants : 1) les limites spatiales et les fluctuations temporelles de la zone d'impact; 2) les zones de référence pour chaque type de substrat ou d'habitat touché; 3) la cause et l'ampleur des changements environnementaux; 4) les prévisions des tendances que présenteront les impacts.

Pour définir l'étendue spatiale d'une zone d'impact, il faut pouvoir faire des comparaisons entre les conditions avant et après les impacts ou entre des conditions témoins et les conditions d'impact. Il est important de recueillir des données références pour établir les conditions « avant » dans les secteurs qui seront touchés, ainsi que des données caractérisant les conditions « témoins » dans des secteurs qui ne seront pas touchés par des impacts à distance sur le milieu benthique. Pour établir des secteurs « témoins » ou de référence, il faut avoir une connaissance pratique de l'hydrographie, du substrat, de la bathymétrie et des caractéristiques de l'habitat au site touché de façon à bien comparer le milieu témoin et le milieu touché. Le choix des paramètres physiques, chimiques et biologiques à surveiller déterminera largement la capacité de détecter des changements environnementaux. Les propriétés à mesurer devraient comprendre 1) des traceurs de déchets (p. ex., le zinc), 2) des paramètres chimiques et physiques altérés par l'apport de déchets (p. ex. les sulfures et le potentiel redox) et 3) les réactions biologiques aux apports directs de déchets et à leurs sous-produits. Des études approfondies présentant des exemples pratiques de données de comparaison de la variabilité spatiale et temporelle entre des sites touchés et des sites de référence dans la Région du Pacifique sont nécessaires pour établir une hiérarchie des paramètres de surveillance selon leur capacité de détecter les impacts écologiques.

1.0 Introduction

The purpose of this paper is to review and recommend suitable sampling designs and methodologies for a benthic monitoring program in the Pacific Region suitable for detecting environmental changes associated with salmon aquaculture. In general, benthic monitoring programs designed to detect environmental change require the following aspects to be defined: 1) spatial boundaries and temporal fluctuations of impact zone; 2) reference zones for each impacted substrate or habitat type; 3) cause and magnitude of environmental change; and 4) future predictions of impact trends. Sampling designs and variables to be measured will be presented with these aspects in mind following two themes: a far-field long-term approach and a near-field short-term approach. In addition, a decision tree is presented to outline recommended methods and variables when bottom types vary from mud to rock on flat to sloped topography, since no single monitoring technique or sampling method is suitable for all bottom types.

In terms of the PSARC request, the 6 sub-questions were not addressed independently, since such an approach would not provide the continuity required by a monitoring program that spans across various substrate types, depths, and inclines and requires a hierarchical statistical approach. Furthermore, employing a multitude of sampling methods and statistical analyses specific to delineated environments and different applications (baseline data, mitigation and monitoring, authorization conditions) will ultimately increase resource requirements and, thus, the "cost-effectiveness" of a monitoring program. In order to provide a scientifically-defensible and practical monitoring program, a comprehensive framework has been presented that outlines a hierarchical approach to methods and analyses.

The overall goal of this PSARC request focuses around the need for an early warning system for a potential HADD (Harmful Alteration Disruption and Destruction). It is difficult to accomplish this goal since 1) no definition or threshold exists for a HADD and 2) our confidence in identifying "significant habitat alteration" exists at a gross level of sulphide concentrations (6000 μM) outlined in a DFO-recommended impact classification scheme (Wildish et al. 1999). In order to determine an early warning system for a potential HADD, one must incorporate a technique with a high resolution capability and have a good working knowledge of background levels of the parameter of interest. In addition, local groundtruthing is required in order to adopt recommended monitoring tools verified in other parts of the world (Levings et al. 2002). Thus, at this point in time an early warning system for a potential HADD can not be put in place but may be developed from knowledge gained from monitoring and research programs. In order to define a HADD the following questions should be answered: 1) what level of ecological alteration is required to take place, 2) how long should this ecological change be sustained for?, and 3) over what area should this ecological change take place?.

2.0 Considerations for a long-term (far-field) monitoring program

2.1 Determining aerial extent of impact zone

In order to define the spatial extent of an impact zone, comparisons of before and after conditions and/or control and impact conditions should be considered (Green 1979; Underwood, 1994). It is important that baseline data in support of "before" conditions be collected in regions that will receive impact, while data characterizing "control" conditions be collected in regions that will remain uninfluenced by far-field benthic impacts. Far-field impacts can take place as 1) a chronic accumulation of waste material through deposition or benthic transport or 2) the acute transport of waste material at an offshore sedimentary sink (Frid and Mercer, 1989). As a result, it is suggested that the spatial extent of monitoring programs extend well beyond an aquaculture lease area to encompass both near-field and far-field effects, while including well-defined reference areas. For example, Brooks et al. (2003) showed that effects of organic enrichment from an aquaculture operation could be seen at distances sometimes greater than 200 m from the netpen system. This study also documented the Near-Field remediation of zinc-enriched sediments via benthic dispersion and/or the remineralization of zinc-bearing organics. Thus, the recovery of the near-field sediments may have reflected merely the redistribution of Zinc-rich sediments, leading to potential secondary far-field effects. In this example, the spatial extent of benthic monitoring was not designed to adequately assess the gradient of perturbation in the impact zone, nor assess the nature of potential far-field effects. Monitoring over a large area will also allow for the application of statistically nested sampling designs incorporating multiple spatial scales, since disturbances may not have a similar impact across all spatial scales (Bishop et al. 2002). Thus, some knowledge of the local bathymetry and spatial extent of the potential impact is required to ensure that sampling designs allow for the representation of adequate spatial scales and baseline information.

Determining the spatial extent of benthic impacts and defining sufficient reference areas can be carried out using continuous, semi-continuous, and discrete mapping techniques. Continuous mapping techniques consist of multibeam surveys which provide a high spatial resolution assessment of the seafloor and benthic habitats, while discrete mapping techniques consist of spot grab/core sampling and may provide a non-representative distribution of benthic habitats. Since variations in seafloor substrate type are typically linked to variations in benthic community compositions as well as the type of environmental change (Auster and Langton, 1999), ground-truthed acoustic surveys can be used to identify benthic habitats (Magorrian et al. 1995; Wildish et al. 1998; Kostylev et al. 2001) and define the boundaries between impacted and non-impacted areas under patchy conditions. A mapping area extending to 1 km beyond the lease site may be appropriate when taking into consideration the existing siting criteria.

Semi-continuous mapping techniques consist of acoustic (QTCView) and video (ROV) surveys involving spaced transects within a grid system. Although semi-continuous and discrete sampling techniques can be used if continuous mapping methods are not available, ideally, the ROV surveys and grab sampling should be used to ground truth acoustic surveys. The extensive aerial coverage obtained by acoustic surveys over large depositional fields will provide a steering platform for designing smaller-scaled, cost-effective monitoring programs that can better target critical benthic habitats and reference areas identified within a seafloor survey.

Monitoring programs must incorporate a flexible sampling design if the location and orientation of the waste source varies spatially or temporally. The movement of netpens within a lease site, which may can occur due to changes in production strategies, will cause difficulties when defining spatial enrichment as well as fallowing gradients. For example, a control station representing "before" conditions may be rendered inaccessible by sampling equipment if a netpen system is relocated to this site, while a Far-Field station may experience Near-Field conditions if a netpen system is moved from its previous or central lease location. In addition, tidal movement of netpen systems may also impact the results of closely spaced near-field stations (e.g. 0 and 5 m apart) given the potential for large lateral gradients over metre-scale distances in the primary impact zone. As a result, a statistical analysis that relies on repeated measures at specified station locations will not be robust if the effective distance between sampling stations and the waste sources changes. This situation may be amplified if sampling conditions decrease ones ability to get back on station and the benthic environment exhibits a high-scale of patchiness.. Thus, it is important that changes in netpen arrangements as well as the location of waste inputs are well documented to help interpret fluctuations in benthic community response curves to organic enrichment along monitoring transects. The fact that a lag in the benthic community response may take place relative to detected changes in sediment chemistry from waste feed or faeces must also be considered.

The need for program flexibility also relates to the style and orientation of netpen systems, which can vary from site to site or between production cycles within a site. For example, the polar-circle net-pen systems tend to be spaced apart across a lease site, while the square-cage systems tend to be concentrated in a coupled-row pattern and located within a specified region of a lease site. Consequently, defining the "centre" of the waste source may be difficult. Sampling under or between the two types of netpens of similar waste inputs may provide different results given that netpen-netpen interactions will be prevalent in the concentrated square-cage setup. Given that sampling under netpen systems is essential in order to address all potential impacts in regards to determining a HADD, comparisons of the level of impact between the two types of netpen systems should be standardized to account for variations in waste dispersion patterns and overlapping net-pen interactions. In addition, the design of a fixed radiating sampling grid applied to both types of netpen orientations may not be feasible if station transects associated with dominant tidal directions may not consistent if transects are obstructed by cage orientations.

2.2 Defining reference areas

In order to identify "control" or reference areas one must have a working knowledge of the hydrography, substrate, bathymetry, and habitat characteristics of the impact site in order to provide a proper comparison of the control and impact environments. As outlined above, multibeam surveys can provide high spatial resolution mapping of an extensive region of interest and help identify existing substrates or habitats that need to be represented in reference sites. Archambault et al. (2001) argue that the use of more than one reference site is essential when measuring the recovery of a benthic assemblage following the cessation of waste outfalls. The patchy and steep-sloped nature of the near-shore environment where farms are located in the Pacific Region will likely harbour a variety of habitats within a single lease site, suggesting that multiple reference sites or habitats be incorporated into sampling designs. The need for multiple reference habitats may vary depending on the heterogeneity of a site, which again emphasizes the need for flexibility within a monitoring framework. In addition, it is also important to 1) determine estimates of natural variability of "before" as well as "control" conditions in order to isolate the impact signal from seasonal noise (Green 1979; Underwood 1994) and 2) choose reference sites that will not be impacted by other anthropogenic sources (i.e. cumulative effects).

Reference stations serving as the endpoint of a continuum of stations standardized by bathymetry and substrate (i.e. gradient-oriented station selection) may provide more information as to the cause of environmental change than reference stations selected based solely on specific distances from a designated impact site (i.e., grid pattern). In terms of statistical analyses, the former sampling design is conducive for regression analysis, while the latter sampling design is suited for ANOVA analysis. The results of an ANOVA test can examine whether the control/before conditions are different from the impact conditions. However, such statistical assessments may not yield sufficient process-related information to assess cause and affect relationships. Conversely, a sampling layout designed to support a regression analysis will result in developing a response curve outlining the relationships between the sediment chemical constituents and benthic assemblages which may identify correlation or cause. Depending on the nature of the regression curve habitat alteration thresholds may be identified within these response curves. Furthermore, those response curves characterized by high correlation coefficients may be used to identify chemical triggers which may serve as monitoring tools.

In a situation where aerial coverage is emphasized relative to spatial replication, it is suggested that a "repeated measures" approach be adopted for detecting environmental impact (Green, 1993). However, it is very difficult to re-sample at a designated benthic station within an oceanographic setting given variations in the 1) capabilities of positioning technologies, 2) boat drift, 3) wire angle of deployed instruments even under ideal weather conditions; and 4) temporal variability in tidal and regional currents. As a result, spatial variation will exist within the repeated measures approach especially in scenarios where the benthic environment is characterized by metre-scale heterogeneity. A high variation within replicates will reduce the ability of an ANOVA to detect

differences. Furthermore, an ANOVA that tests a sampling design with a high spatial coverage and low replication will not be very robust. Alternately, the regression analysis is not dependent on "repeated measures" as the variation observed at each station and across stations is normalized in the expressed correlation linking the sediment chemical constituents with infaunal assemblages. Multivariate analyses (Prinicipal Component Analysis) drawing correlations between sampled parameters independent of station location will be very useful in directing the use of regression analysis for a flexible monitoring program

2.3 Detecting the cause and magnitude of environmental change

The selection of the appropriate physical, chemical, and biological parameters to be incorporated into a monitoring program will largely influence the capability to detect environmental change. Properties to be measured should include 1) tracers of waste material (ex. Zinc), 2) altered chemical and physical parameters resulting from waste input (ex. Sulphides/Redox), and 3) biological responses to the direct waste inputs and indirect byproducts. The parameters put forward in the paper for monitoring aquaculture waste are based on their value in enhancing the level of detection of organic enrichment and not on their feasibility to be measured by consultants and/or industry technical staff. If multiple groups are involved in collecting measurements then a standardization of analytical methods for the recommended monitoring parameters is required to maintain the integrity of the program. Once a hierarchy of ranked parameters is established based on the contribution to impact, a suite of "triggers" can be selected for future monitoring efforts. Setting limitations on monitoring parameters in the early stages of knowledge acquisition will compromise the ability to properly estimate impacts and may result in an overall underestimation of habitat alteration.

In order to provide continuity to a monitoring program, it is important to choose parameters that can be measured across substrate or medium types (Figure 1). Although sediment porewater constituents (sulphides, oxygen, trace-metals) cannot be measured in the veneer or crevices of a rocky boulder substrate or a shell-sand substrate where the deployment of traditional grab and core samplers are not possible, estimating these constituents in the associated near-bottom water environment will be a valuable alternative. Sediment-water exchanges of constituents may provide information regarding the influence of impacted sediments as well as rocky substrates on the overlying water and allow us to estimate the potential for sediment remediation knowing efflux rates of sediment constituents.

The following monitoring parameters outlined below represent potential indicators or triggers for detecting habitat alterations. Further research providing practical examples of data that compares spatial and temporal variance at reference/impacted sites within the Pacific Region is required in order to create a hierarchical ranking of monitoring parameters according to their ability to detect ecological impact. Currently we are struggling with a question posed by Underwood (1994), "Is there a better way to capture the signal of environmental change in the noise of ecological variation". Determining a means of capturing a signal involves incorporating factors other than

detection ability such as cost-effectiveness, practicable, and reliable. For example, an assessment of the cost-effectiveness of a short-term monitoring program will be biased towards initial cost of equipment, while that of a long-term monitoring program will be biased towards ongoing costs associated with equipment maintenance or sample analysis. A wide suite of equipment will be required in a monitoring program that is designed to sample a wide range of substrate (mud/sand/rock) and habitats (eelgrass/kelp) thereby affecting the initial cost of a monitoring program. Factors affecting the analytical costs of a sampling program will without doubt be the level of taxonomic resolution incorporated into faunal analysis. Although several studies have indicated that a lower taxonomic resolution (family level) be considered for monitoring fish farm impacts (Karakassis and Hatziyanni, 2000) or for describing natural spatial variation of fauna (James et al. 1995), these findings should be confirmed across all substrate types and levels of impact within the Pacific Region. In addition, it will be a challenge to find a balance between "practical" and "reliable", since most methodologies are initially screened based on their ability to be carried out in remote places on small boat operations in the Pacific region.

2.3.1 Monitoring parameters for soft substrates

Certain monitoring parameters are chosen based on their direct link to feed and faecal waste (ex. zinc), while others are chosen based on their indirect link to organic enrichment and effect on biological assemblages (ex. Dissolved sulphides). While the method of sample acquisition for each parameter is outlined in this paper, the analytical analysis of each sampling component normally carried out at contract laboratories is not. This topic warrants a review for further standardization of an aquaculture monitoring program.

2.3.1.1 Physical properties:

An analysis of **sediment grain size (SGS)** is an important monitoring parameter, since the deposition of fine particles may be enhanced in association with fish farm operations as flow rates tend to decrease through netpen systems (Inoue, 1972; Weston 1986). **Sediment Porosity (SP)** is a measure of water content and has been shown to be elevated in the surface sediments associated with fish farms relative to that of the control site (Karakassis et al, 1998). Since water content is typically high in unconsolidated sediments (Maa et al. 1997), it may serve as a sensitive monitoring parameter within fish farm deposits characterized by high sedimentation rates. Water content is defined as the percent mass difference between wet and dried sediment samples. **Bulk density (BD)** is also a measure of consolidation of sediment and organic material within the seabed. Since sediment compression can take place during the extrusion of a sediment sample from a core barrel, estimating the mass of material within a specified volume may be challenging. High-precision techniques and coring methods are necessary to obtain accurate bulk density values especially when dealing with unconsolidated gel-muds (Amos et al. 1996, Sutherland et al. 1998a). For methods of sample collection for the above parameters see Table 1.

Table 1: Sample collection of physical, chemical, and biological parameters and processes within soft sediments.

Sampling equipment	**Sediment source**	**Monitoring parameter**	**Volume/area collected**
Still photograph	Sediment surface	Biofilms, Epifauna, Epiflora Colour of sediment	1 x 1.5 m
Van Veen Grab Area: 0.1 m^2	Top 2 cm scrapings	Sediment Grain Size Sediment Porosity Organic Content Total Organic Carbon Total Nitrogen Zinc, Copper, Lithium	250 ml 100 ml 100 ml 100 ml
Van Veen Grab Area: 0.1 m^2	Vertical subcore	Meiofauna Chlorophyll Bulk Density	60-cc syringe core 60-cc syringe core 60-cc syringe core
Van Veen Grab Area: 0.1 m^2	Entire grab	Macrofauna	18 Litres
Gravity core	Insert probe into side port of core barrel	Redox Potential Free Sulphides Oxygen Content	N/A
Gravity core	Collect subsample from side port of core barrel	Ammonia Total Organic Carbon Total Nitrogen Colour	10-cc syringe core 10-cc syringe core
Benthic chamber	Deployed on sediment surface	Benthic respiration	N/A
Dialysis Arrays (Peepers)	Deployed across sediment-water interface	Dissolved pore-water constituents (S, NH_3, metals)	5-10 ml per horizon

*may require SCUBA deployment

2.3.1.2 Chemical properties:

Particulate parameters: **Organic content (OC), Total organic carbon (TOC), and Total Nitrogen (TN)** have been shown to exhibit spatial and temporal trends in association with the deposition of fish farm wastes (McGhie et al. 2000). Organic content is defined as the percent difference between dried and ashed sediment samples. In terms of metal constituents, **zinc** and **copper** have been shown to exist in feed pellets (Lorentzen and Maage, 1999; Sutherland et al. 2001) and to accumulate in impacted sediments underlying fish farms (Uotila, 1991; Chou et al. 2002). The standardization of zinc and copper data using a lithium-normalization technique has been suggested in order to identify metal ratios that reflecting anthropogenic inputs (Loring, 1991; Yeats et al. 2004). It is recommended that monitoring programs adopt this technique and include the

measurement of **lithium**, since this method will serve as a useful tracer for fish feed waste. For methods of sample collection for the above parameters see Table 1.

Pore-water parameters: Changes in **Redox potential (Eh)** have been shown to be related to organic enrichment (Hargrave et al. 1997) and fallowing processes (McGhie et al. 2000) associated with fish farm activities. In addition, high levels of **Total free sulphides** (hydrogen sulphide (H_2S), hydrosulphide anions (HS^-), and bisulphide anions (S^{2-}); Wang and Chapman, 1999) have been observed in impacted sediments of fish farm operations relative to control areas (Hargrave et al. 1997; Brooks et al. 2003). Dissolved **oxygen content** decreases in sediments in association with the degradation of accumulating organic matter (Jorgensen, 1983). To date the technique suggested by Wildish et al (1999) has been used in the Pacific Region by DFO Scientists with two exceptions: 1) sediment cores are collected using a gravity corer (Pedersen et al. 1985) as opposed to SCUBA methods and 2) an oxygen probe is initially inserted into each sampling port of the core barrel to obtain an oxygen measurement. Nilsson and Rosenberg (1997) have developed a profiling imaging system designed to provide information on sediment texture, oxic/anoxic conditions, and lamination as well as faunal burrows, tubes, and bioturbation.

The Scientific Advisory Group (2001) suggested that sediment **ammonia**, which is widely recognized as a major toxicant, be considered as an indicator of fish farm impact. In the absence of an available probe designed to detect ammonia, sediment porewater constituents can be determined by the following approaches: 1) centrifugation of sliced sediment cores (Sholkovitz, 1973); 2) pressure filtration of sliced sediment cores (Presley et al., 1967); 3) expulsion of porewater through sampling ports from pressurized intact sediment cores (Jahnke, 1988); 4) collection porewaters obtained by suction of sediment slices (Sayles et al., 1973); and 5) in situ sampling using diffusion-based dialysis samplers (Hesslein, 1976). All of these techniques work best in finer grain size environments (*e.g.*, clays, silts, fine sands), and are not suited for coarser or steeply-sided substrates.

2.3.1.3 Biological properties:

Microbial biofilms: The distribution of benthic bacteria and microphytobenthos (benthic diatoms) appears to be influenced by fish farming activities (La Rosa et al. 2001). Furthermore, bacterial mats (*Beggiatoa* sp.) can dominate in seafloor areas where the sediment surface is blackened and mounded with waste food (O'Connor et al. 1993). Still photography can be used to visually determine the percent cover of microbial mats, while 60-cc syringe cores can be used to collect vertical profiles for quantitative determination of chlorophyll-a (microphytobenthos). The Photographic unit of the Geological Survey of Canada - Atlantic designed and built a submersible 35 mm still camera mounted on a frame with a flash unit and a trigger weight release mechanism that can be used to collect pictures of microbial mats covering the seafloor (Amos et al. 1997). Photographs are taken at a height of about 1 m off the bottom and cover an area of 1 x 1.5 m.

Macrofauna (> 500 microns): Changes in macrofaunal communities in response to organic enrichment associated with fish farm operations have been documented (Ritz et al. 1989). Macrofauna samples can be collected using a 0.1 m^2 Van Veen Grab and sieved through a 0.5 mm sieve. A 1.0 cm sieve is commonly used to pre-screen the sample to reduce clogging events and facilitate the sieving process through the finer mesh (0.5 mm). Eleftheriou and Holme (1984) suggest that a 0.5 mm sieve be used for macrofaunal collection, since the retention of macrofauna on a 0.5 mm and a 1.0 mm sieve have shown an underestimation of certain macrofauna a 1 mm mesh (Lewis and Stoner, 1981; James et al. 1995). Sieved samples are then preserved in buffered 10% formalin. It is important to note that epifauna and macrofauna will be collected simultaneously when sediment samples are collected using a Van Veen grab.

Meiofauna (63 – 500 microns): It has recently been demonstrated that the deposition of fish farm wastes has a strong impact on meiofaunal assemblages (Mazzola et al. 1999; La Rosa et al. 2001; Mirto et al. 2002). Duplisea and Hargrave (1996) suggest that meiofaunal sensitivity to environmental impacts in general may be useful in detecting initial changes of the benthos due to fish farm biodeposits. The increase in interest in the use of meiofauna as an environmental tool is due to their small size, lack of larval disturbance, and high reproductive rate (Higgins and Thiel, 1988, in Mirto et al. 2002). Meiofauna samples are collected using modified 60-cc syringe cores inserted into a Van Veen grab containing a sediment sample. The cores are then extruded and sectioned at 1 cm intervals, and preserved in 4% formalin. The mieofaunal fraction is then extracted from the sediment sample following the method of Warwick and Buchanan (1970).

2.3.1.4 Exchanges across the sediment-water interface

Measuring processes at the sediment-water interface may provide a more powerful estimate of impact than collecting discrete samples, since processes incorporate the recycling component of constituents where discrete sample provide only a "snapshot" of ambient concentrations. Measuring sediment processes such as benthic respiration will also provide an indication of the assimilative capacity of sediments associated with fish farm deposits. Estimates of benthic respiration can be determined using a bell jar chamber (Hargrave and Connolly, 1978) which would aid in assessing the effects of benthic organic enrichment.

The quantification of concentration gradients across the sediment-water interface can be used to estimate the diffusive transfer of contaminants across the benthic boundary (Pedersen et al., 1984). Specifically, the porewater distribution of contaminants of concern (zinc, ammonia), in conjunction with other interstitial metabolites (dissolved iron, manganese, sulphides), can be used to infer the redox processes governing contaminant mobility and behaviour (Klinkhammer et al., 1982). Reviews of these various porewater methodologies indicate that dialysis techniques present the most reliable and artifact-free method (Brandl and Hanselmann, 1991; Carignan et al., 1994). Dialysis arrays (peepers), which sample pore waters passively by allowing equilibration of water-filled peeper cells with adjacent pore waters, operate on the principle of

molecular diffusion. Peepers provide profiles of filterable constituents typically from ~20 cm above the benthic boundary to a sub-interface depth of ~30 cm, potentially penetrating into a pre-aquaculture layer. Peeper deployment can be performed by SCUBA diver insertion, or conversely, via the use of benthic landers (Martin and Pedersen, 2002).

2.3.2 Monitoring parameters for near-bottom waters

Knowledge of the sedimentation, resuspension, and transport of waste particles in the near-bottom waters will allow one to accurately determine rates of organic enrichment and fallowing processes in sediments associated with fish farm operations. In addition, estimates of near-bottom particle fluxes will allow one to determine the effect of impacted sediments on the overlying water column or far-field environments. Monitoring the near-bed region will provide another means of identifying direct (tracer) and/or indirect (organic enrichment byproducts) effects of fish farming activity, if exchange processes (diffusion or resuspension) of both particulate and dissolved constituents occurs across the sediment-water interface and if sediment/waste samples are not retrievable from coarse substrates or rocky crevices. The presence of high sulphide and low oxygen porewaters within impacted sediments may influence organisms residing in the benthic boundary layer, since hydrogen sulphide (H_2S) is toxic to fish (Bagarinao, 1992) as well as benthic invertebrates (Theede, 1972).

2.3.2.1 Physical, chemical, and biological properties

In order to provide continuity to a monitoring program and address the effect of fluxes of constituents across the sediment-water interface, it is important that near-bottom water measurements mirror those collected in soft and hard substrates environments. In general, particulate and dissolved constituents within the near-bed can be measured on relatively flat bottom types using profiling landers such as the Benthic Organic Seston Sampler (Boss; Muschenheim and Newell, 1992) and the BIOPROBE, Biological Processes Bottom Environmental (Thomsen et al. 1994). BOSS collects discrete water samples simultaneously at 10 heights within the 0.5 m of the bottom. The water samples can then be filtered for particulate analysis including **Sediment grain size**, **Suspended particulate matter**, **Organic matter**, **Total organic carbon**, **Total nitrogen**, and **Trace metals**, while the filtrate can be analyzed for dissolved constituents (**Sulphides**, **Eh**, **Oxygen**, **Ammonia**, and **Trace metals**). Physical properties such as Salinity and Temperature can be measured from the filtrate samples.

Bioprobe collects 4 water samples (5, 10, 20, and 40 cm above bottom) and is equipped with a camera, flow metre and optical backscatter senser. This combination of instruments would provide information regarding the thresholds and frequencies of resuspension events. However, the larger size and weight of BIOPROBE relative to that of BOSS may make deployment from small boat operations cumbersome. The sedimentation fluxes specific to tidal directions can be measured using a SMARTRAP (Steeves et al. 1993), while erosion and benthic transport measurements can be measured using a Sea Carousel (Amos et al. 1992; Sutherland et al. 1998a). As mentioned in

section 4.1.4, benthic respiration and fluxes of dissolved constituents can be measured in soft substrates using a bell jar chamber (Hargrave and Conolly, 1978) and a peeper unit (Martin and Pedersen, 2002). Collecting near-bottom water samples for sloped substrates, where the deployment of landers is not possible, can be achieved using bottle, pump, or pipe samplers (Sutherland et al. 1992).

2.3.3 Monitoring parameters for hard substrates

2.3.3.1 Physical, chemical and biological properties:

The nature of a hard-bottom seafloor can be identified through the bathymetry and backscatter signals provided Multibeam acoustic surveys. Details regarding the substrate types can be verified using groundtruthing techniques (Clarke et al. 1996) and provide information pertaining to whether the rock surface is smooth or bouldery and whether or not sedimentary sinks exit between boulder interfaces. This information can be used to steer a monitoring program targeting certain substrate or habitat types and identifying sampling equipment requirements.

Still photographs and video recordings can be used to provide calibration information for acoustic surveys across designated stations or transects or as an independent survey to quantify waste material, epifaunal abundance and/or epiflora cover (Crawford et al. 2001). Drop cameras or remotely operated vehicles will have to be used as water depths within the vicinity of fish farms in the Pacific region typically extend beyond those compatible with SCUBA. When considering a flat-bottom, hard substrate ROV video recordings can be carried out along specified transects. In a situation involving drag from high currents, video transects can be accomplished by towing a suspended small-scale ROV in a certain direction (Starmans et al. 1999; Collie et al. 2000). Towed video cameras have been deployed successfully along transect lines as part of an assessment study of fishing gear impacts (DFO:ESSRF). In this study, real time video coverage was viewed from the boat deck allowing the operator to maintain a constant height above a grading seafloor sloop as well as the focal point. Drop cameras can be deployed at regular intervals or stations along specified stations or attached to ROV frame. Collie et al. (2000) found that less detail was visible in video recordings (Hi-8 mm) relative to still photos collected during an impact assessment due to fish gear. Recent technology incorporated DVD-quality video may resolve the clarity issue of video images.

When considering a steep-sloping, hard bottom-type, video recordings of straight transects may become difficult to achieve. Although a leap-frog technique may be employed as a camera climbs a steep slope, a considerable amount of the video recording may be rendered useless as the camera cannot maintain a constant focal point or image area. Drop cameras (Amos et al. 1997) equipped with trigger weight release mechanisms may provide a standard method (image area) of obtaining a picture of the seafloor if the camera is always activated at a set distance above the substrate (1 m). Video or camera analyses should provide quantitative estimates of **feed pellets**, **faecal matter**, **sediment colour**, **microbial cover**, **epifloral cover**, and **epifaunal abundance** (Crawford et al.

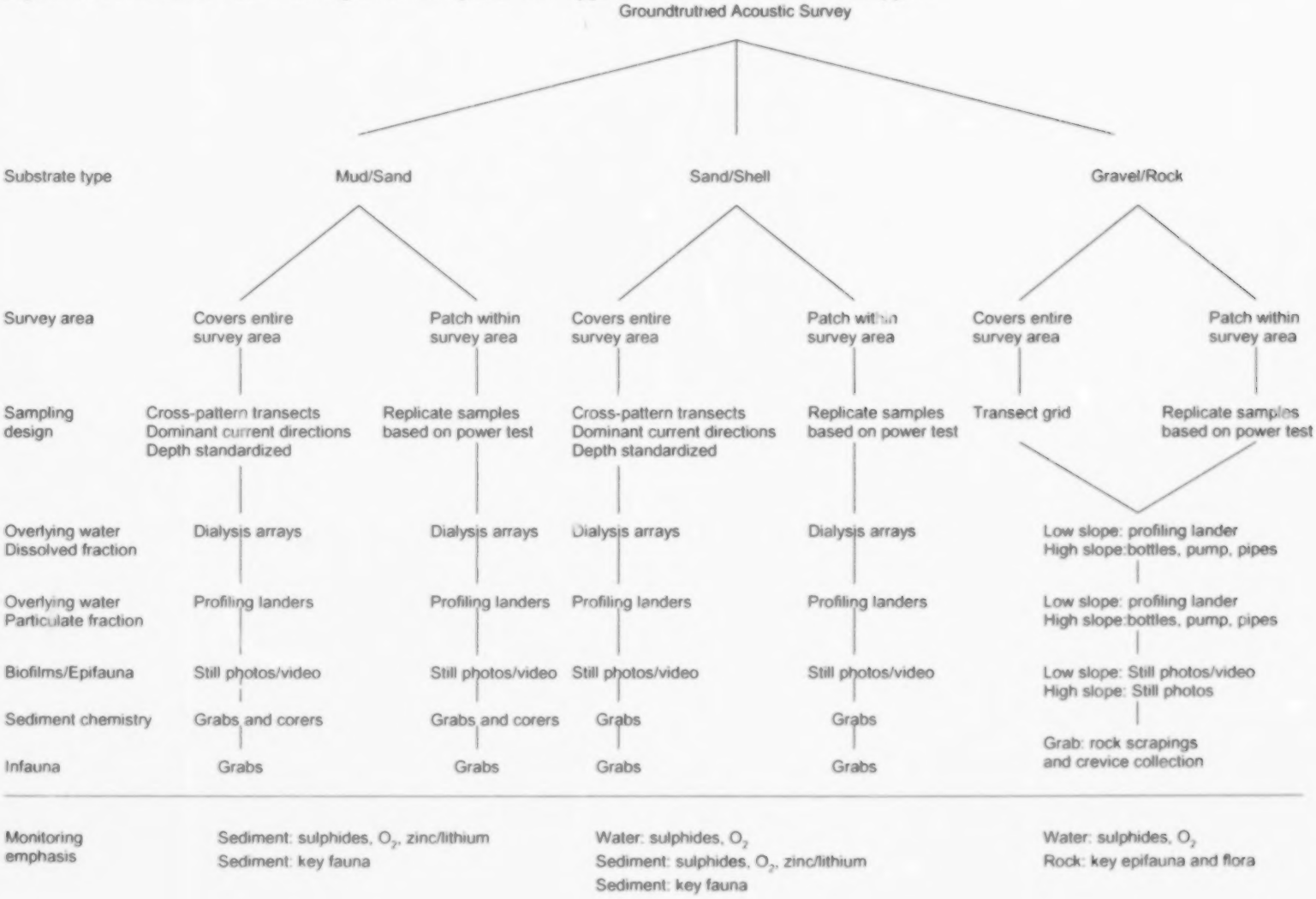
2000). Digitization of frozen video frames using software packages (ex. OPTIMUS) can be use to estimate aerial coverage of targeted flora or fauna using filters (Sutherland et al. 1998b).

Sediment or biological veneers overlying a rock substrate may be collected by carrying out a grab or dredge scraping of the soft material. Particulate analyses for parameters outlined in section 4.1 could be carried out if enough material is collected.

3.0 Sampling design for homogeneous and heterogeneous substrates

Figure 1 outlines sampling designs, monitoring equipment, and sampling parameters suitable for different substrate types. An acoustic survey is initially required to identify the substrate/habitat type and degree of patchiness of the area of interest. When considering a homogeneous soft-bottom survey area, a cross-pattern of transects would be suitable to use with an effort made to locate transects within the dominant current direction and along single depth contours. This sampling design may be possible since most soft-bottom substrates tend to be characterized by a low sloping grade. When considering a homogeneous hard-bottom survey area, a transect grid pattern would be suitable to use with an effort to locate transects within the dominant current directions. Standardizing transects according to water depth would be difficult in this environment, since most extensive hard-bottom regions are located on steep slopes. The spacings of video transects and image areas of still photographs require calibration in order to determine that an adequate number of the existing epifauna or cover of epiflora have been documented to provide proper population estimates. This calibration may involve varying the video transect widths, quadrat areas, and/or photograph image areas over a specified region of each habitat type (Barbeau et al. 1996; Hatcher et al. 1996). The data from the substrate-specific calibrations of video/photo images at each farm site can be compiled into a master file to formulate future sampling designs of known substrate types. When considering a high degree of substrate patchiness within the area of interest, a power analysis of spatial coverage and sampling frequency of each monitoring parameter should be carried out for each habitat type.

Figure 1: A decision tree outlining monitoring methods applicable to various substrate types.



4.0 Considerations for a short-term (near-field) monitoring program

Since it is technically difficult to delimit precise geographic zones of impact, sampling should be restricted to transects defining an organic enrichment gradient along dominant current directions. A cross-pattern of transects can be employed through the centre of the fish farm location with two transect axes running along the major current directions. Replicate samples are collected at 0, 30, 100, and 300 metres along each transect. Reference stations at fish farm sites located within a homogenous environment (one habitat type) can exist at the end of a sampling transect that spans the lease site (depth standardized). Additional reference sites can exist in the far-field regions (not within the sampling transect) under conditions of similar water-depth, substrate type, and hydrographic conditions. These reference sites will be of particular significance in high-slope environments where the transect endpoints (reference sites) may not serve as an appropriate control for the near-field stations. Reference stations at fish farm sites located within a heterogeneous environment (multiple habitat types) should include a set of replicate stations for each habitat type (similar water-depth, substrate type and hydrographic conditions).

Sampling parameters for soft substrates should include 1) a tracer of waste material (Zinc:Lithium ratio), 2) a trigger reflecting organic enrichment, and 3) a faunal component (macrofauna > 0.5 mm). A suite of triggers consisting of sediment grain size, sediment colour, organic content (TVS), water content (porosity), organic carbon, nitrogen, sulphide and Eh measurements should be collected. A full Van Veen grab is used for macrofauna collection, while the uppermost 2 cm of the Van Veen grab is used for sediment chemistry parameters (triggers). A gravity core is used to obtain vertical profiles of sulphide and Eh every 2 cm. Sampling parameters for hard substrates should also include 1) a tracer of waste material (feed pellets and faecal material), 2) a trigger reflecting organic enrichment (substrate colour, vaneer scrapings, and bottom water analysis, and 3) a faunal component (microbial, epifloral, and epifaunal cover). Sampling methods for hard substrate environments should include remotely operated vehicles (ROV), towed videos, and/or bottom drop cameras. Since a paucity of data exists regarding the criteria for video survey methods and video analyses for a variety of substrate types and faunal/floral assemblages, on-site calibrations are required to provide a confidence level in substrate classifications and population estimates of certain species. Calibrations involve varying video transect widths, quadrat areas, photograph image areas, number of frames analyzed, number of species counted for each survey (Barbeau et al. 1996; Hatcher et al. 1996).

It is important to recognize that the short-term monitoring program is limited in that it is biased toward detecting a HADD within soft substrates as methodologies have been worked out and limited to gross determinations of HADD along restricted sampling regions. Since sampling transects may not span across all habitat types existing within the entire impact zone, the short-term monitoring program may not address the Fisheries Act concerned with impacts to all types of existing fish habitat and CEAA reviews concerned with siting criteria regarding sensitive fish habitats.

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